ents and oxygen. Micro-mixing pertains to mixing in an area of 1 to 10 cell diameters, while macro-mixing means mixing of the entire liquid volume in the container.

According to one aspect of the subject invention there is provided an improved fermentation vessel for propagating animal cells in suspension cultures and monolayer cultures in which oxygen must be supplied to the cells in a liquid nutrient medium in the vessel for cell metabolism and multiplication, with the improvement comprising a permeable membrane, in the fermentation vessel, through which oxygen can diffuse directly into the liquid nutrient medium containing the cells.

According to a second aspect of the invention, there is provided an improved method of supplying oxygen to animal cells growing in suspension cultures and monolayer cultures in a fermentation vessel containing a liquid nutrient medium, comprising passing the oxygen through a permeable membrane in the vessel so that it diffuses directly into the liquid medium and thereby enriches it for the benefit of cell multiplication.

It was found, surprisingly, according to the invention that all of the oxygen needed for propagating animal cells in culture suspensions, or monolayer cultures, in fermentation vessels can be supplied by having the oxygen pass through a permeable membrane into the liquid. No additional supply of oxygen is needed and, in particular, the bubbling in of air is unnecessary and, thus, the disadvantages associated with that method are avoided. The method of the invention avoids the shearing forces caused by bubbling in a stream of air or oxygen, eliminates or substantially reduces foam formation, and avoids the prior art problem of correctly sizing the holes through which the air bubbled.

A very important advantage of the invention is that now, for the first time, propagation of animal cells, and particularly insect cells, is possible in culture suspensions and monolayer cultures in fermentation vessels in much larger volumes than was previously possible or customary. Thus, because of the invention is to possible to produce or ferment culture suspensions of ten liters or more with the cells multiplying at a maximum rate. Fermentation volumes can now be produced of 15 to 20 liters or more, which were not previously possible, and volumes of 50 and even 100 liters are not considered impossible.

The material used for the permeable membrane must be one which permits an adequate amount of oxygen to pass through without bubbling. However, the material selected should also be one on which the cells do not grow, or grow only slightly, for otherwise passage of oxygen through the membrane would be impaired and flow possibly reduced thereby leading to an insufficient, or undesirably low, oxygen supply in the liquid medium.

Permeable membranes useful in the invention can be made of any synthetic inert solid polymeric material. Particularly useful are membranes made of silicone rubber, laminated silicone rubber products, and polytetrafluoroethylene (Teflon). Other synthetic polymers can be used provided the animal cells do not adhere to or grow on them. Silicone foil and silicone tubes provide a surface on which the cells do not adhere, or adhere to it only with great difficulty. Therefore, synthetic silicone polymers are preferred. Regardless of the material use, the membrane should be thick enough to provide the necessary mechanical strength but thin enough to permit oxygen to pass through readily. The membrane must, of course, prevent reverse flow through it of liquid from the fermenting broth.

The permeable membrane positioned in the fermentation vessel can have any suitable size or geometric shape but one must be selected so that there is sufficient oxygen diffusion to supply the amount needed for cell metabolism. In addiiton, the permeable membrane size and shape should not interfere with cell propagation in the fermentation vessel. Those skilled in the art will be able to adapt these features, and the material of which the membrane is made, to the customary bio-technological requirements. While the permeable membrane can, itself, completely enclose a space or volume and thus constitute a hollow member, such as when in the form of a tube, sphere or closed pouch, it is also within the scope of the invention to employ a membrane which constitutes only a portion of a chamber wall or surface surrounding a space. In all instances, however, a gas supply conduit means is provided to feed an oxygen-containing gas under pressure so that it can pass from one side of the membrane, through it, and into the liquid nutrient medium on the other side. The gas supply conduit means can comprise a tube extending from outside to inside of the fermentation vessel. In addition, a gas withdrawal conduit means can be included extending from inside to outside of the fermentation vessel. Both conduit tubes should, of course, communicate with a space on the same side of the membrane or with a common chamber or volume wholly or partially defined by the membrane.

A tube or hose, having a wall about 0.6 to 1.2 mm thick and preferably about 1.0 mm thick, wound around a suitable support in the fermentation vessel is particularly useful. The support, for example, can be a heat exchanger such as is customarily used in a fermentation vessel to keep the nutrient medium and culture broth at optimum temperature.

Representative oxygen sources for the fermentation are air, a mixture of air and oxygen, or a mixture of oxygen and nitrogen.

The permeable membrane used for supplying oxygen to the culture growing in the nutrient medium also serves as a filter to remove any microorganisms in the oxygen gas supply stream, particularly when air is used, and keeps them out of the culture broth. This is a distinct advantage since all of the oxygen can be supplied through the membrane.

It should be obvious that, when the fermentation process is carried out according to the invention, the entire apparatus and the nutrient medium used must be sterile.

Providing oxygen by means of a permeable membrane according to the invention results in basically improved environmental conditions for cell culture so it is expected that improved cell multiplication rates will be obtained with a wide variety of vertebrae and non-vertebrae culture suspensions and monolayer cultures.

Vertebrae cell lines of primary importance for propagation according to the invention are those which are used in the mass production of biological products such as immunity factors, hormones, enzymes, anti-viral agents, virus preparations, and vaccines. These include the Psylla (plant lice) cell lines BHK 21, NAMALWA and 1301 cell line (from the leukemia line CCRF-CEMT).